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Evaluation of the U.S. peanut mini core collection using a molecular marker for resistance to *Sclerotinia minor* Jagger

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Abstract Cultivated peanut, the second most economically important legume crop throughout the United States and the third most important oilseed in the world, is consistently threatened by various diseases and pests. *Sclerotinia minor* Jagger (*S. minor*), the causal agent of Sclerotinia blight, is a major threat to peanut production in the Southwestern U.S., Virginia, and North Carolina and can reduce yield by up to 50% in severely infested fields. Although host plant resistance would provide the most effective solution to managing Sclerotinia blight, limited sources of resistance to the disease are available for use in breeding programs. Peanut germplasm collections are available for exploration and identification of new sources of resistance, but traditionally the process is lengthy, requiring years of field testing before those potential sources can be identified. Molecular markers associated with phenotypic traits can speed up the screening of germplasm accessions, but until recently none were available for Sclerotinia blight resistance in peanut. This study objective of this study was to characterize the US peanut mini-core collection with regards to a recently discovered molecular marker associated with Sclerotinia blight resistance. Ninety-six accessions from the collection were available and genotyped using the SSR marker and 39 total accessions from spanish, valencia, runner market types were identified as new potential sources of resistance and targeted for further evaluation in field tests for Sclerotinia blight resistance.

Keywords Mini-core collection · Peanut · Sclerotinia blight · Molecular marker · Resistance

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Introduction

Cultivated peanut (*Arachis hypogaea* L.) is a self-pollinated allotetraploid ($2n = 4x = 40$) and is the second most economically important legume in the U.S. Peanut is susceptible to many pathogens, with most damage being caused by fungi (Melouk and Backman 1995). Soil-borne fungi cause diseases that adversely affect peanut health and productivity throughout the growing areas of the United States. Diseases such as pod rot (*Rhizoctonia solani* Kühn, *Pythium myriotylum*), crown rot (*Aspergillus niger* Teigh) and southern blight (*Sclerotium rolfsii* Sacc) occur in all U.S. peanut-producing areas, while others such as Sclerotinia blight (*Sclerotinia minor* Jagger) are limited to certain geographic regions. Sclerotinia blight is of major concern to peanut producers in the

Southwest US, Virginia and North Carolina. Depending upon severity of field infestation, yield losses due to Sclerotinia blight are typically 10% but may be as high as 50% (Melouk and Backman 1995). Expensive fungicide applications throughout the growing season are often required for effective disease management. Host plant resistance would provide the most effective solution to managing Sclerotinia blight but limited progress has been made in the development and release of cultivars with enhanced tolerance to the disease (Smith et al. 1991, 1998; Baring et al. 2006).

Several factors contribute to the lack of available Sclerotinia blight resistant cultivars. The inheritance mechanism of host resistance is not well understood. Wildman et al. (1992) suggested that at least two loci were involved in Sclerotinia blight resistance among genotypes studied. Cytoplasmic factors have also been suggested to be involved in Sclerotinia blight resistance (Coffelt and Porter 1982). Plant morphology can play an important role in resistance to fungal disease because of the environment required for development and progression (Chappell et al. 1995; Coffelt and Porter 1982; Coyne et al. 1974; Schwartz et al. 1978). Plant types with a more upright growth habit and open canopy, such as spanish, appear to be more resistant than those with a dense canopy (such as runner and Virginia types) which allows for temperature reduction and moisture accumulation. However, the mechanism of resistance among spanish types is not purely morphological since the spanish cultivars Pronto and Spanco are as susceptible as many runner types, suggesting contribution by a genetic component.

Another obstacle for breeding programs developing *S. minor* resistant cultivars is the limited number known sources of resistance. Cultivated peanut has an extremely narrow genetic base which has been explained to have resulted from a single domestication event (Simpson et al. 2001) and subsequent inbreeding among a few select parental lines in commercial breeding programs. The advent of molecular genetics gave peanut breeders new-found hope that undiscovered diverse germplasm would soon become apparent and available for use. However, early examinations of the genetic diversity in cultivated peanut revealed little or no polymorphism among the accessions studied (He and Prakash 1997; Kochert et al. 1991; Stalker and Mozingo 2001). Recently, techniques such as SSR and microsatellite analysis have uncovered some genetic variability in peanut. For example, Hopkins et al. (1999) used simple sequence repeat (SSR) primers to uncover six polymorphic SSRs in cultivated peanut and were able to differentiate 15 of 19 accessions tested. Since that discovery, the number of SSR markers has increased (He et al. 2005). More recently Chu et al. (2007a, b) converted RFLP markers to sequence characterized amplified region (SCAR) markers so as to develop a PCR-based marker system to screen for nematode resistance in peanut and Chenault et al. (2008) discovered a molecular marker associated with Sclerotinia blight resistance.

Molecular markers associated with phenotypic traits have proven extremely useful in breeding programs, either for the characterization of members of segregating populations or for the selection of desired parental genotypes. Markers can also be used as tools for screening germplasm collections for possible new sources of desired traits to be incorporated into adapted lines. In the U.S., germplasm collections exist for most crops and are generally maintained by the Agricultural Research Service (ARS). Germplasm collections are sometimes quite large (over 7,000 accessions in the U.S. peanut collection) and thus core collections containing approximately 10% of the total number of accessions are often developed that represent the phenotypic diversity of the entire collection. For peanut, a core collection containing 831 accessions was developed (Holbrook et al. 1993) and an even more manageable "mini-core" was selected which consists of 112 accessions and is representative of the diversity found in the core collection (Holbrook and Dong 2005). Characterization of this mini-core collection with molecular markers associated phenotypic traits will provide information representative of the entire peanut germplasm collection without the labor intensive and time consuming plight of examining 7,432 accessions individually. The objective of this study was to characterize the U.S. peanut mini-core collection using the molecular marker associated with Sclerotinia blight resistance.

Materials and methods

Plant materials

The mini core subset of the U.S. peanut germplasm collection consists of 112 accessions (Holbrook and

Dong 2005) of which 96 were available and viable and were provided by either R. Pittman (Plant Genetic Resources Conservation Unit, USDA-ARS, Griffin, GA) or by C. Holbrook (Coastal Plains Experiment Station, USDA-ARS, Tifton, GA). Seed from all viable accessions (Table 1) were germinated and grown to maturity under greenhouse conditions. Market type information listed in Table 1 was either taken from Kottapalli et al. (2007) or provided by C. Holbrook (personal communication).

DNA extraction and marker analysis

DNA was extracted from each genotype listed in Table 1, either from dry, mature seed (Chenault et al. 2007) or from young leaf tissue. In case of the latter, 0.2 g of unfolded leaflet tissue was collected from each plant, de-veined, ground in liquid N_2 to a fine powder and vortexed in 1.5 ml extraction mixture [1:1, extraction buffer (0.1 M Glycine-NaOH, pH 9.0, 50 mM NaCl, 10 mM EDTA, 2% SDS, 1% Na-lauryl sarcosine): phenol–chloroform–isoamyl alcohol (25:24:1)]. Extraction mixtures were shaken vigorously for 10 min and then microfuged for 15 min at 10 K rpm at room temperature. DNA was precipitated from the upper layer of each sample by the addition of 750 µl of isopropanol followed by gentle inversion. DNA was spooled onto a glass hook, washed with 70% ethanol, and allowed to air dry for 15 min at room temperature. Hooks were then placed into tubes containing 1 ml extraction buffer and DNA was re-suspended overnight. DNA suspensions were then incubated with 50 µg Proteinase K for 30 min at 37°C. Proteins and other remaining cellular debris were removed by extraction with phenol–chloroform–isoamyl alcohol (25:24:1) followed by extraction with ½ volume of chloroform to remove remaining phenol. DNA was precipitated by the addition of 750 µl isopropanol, spooled on glass hooks and allowed to air dry for 1 h at room temperature. DNA was re-suspended in 100 µl of Tris-EDTA buffer and stored at −20°C until further use.

Amplification using primer pPGPseq2E6L (5′TACAGCATTGCCTTCTGGTG 3′) and primer Marker 3 (5′GCACACCATGGCTCAGTTATT 3′) was carried out in a PTC-100 thermal cycler (MJ Research, Watertown, MA) under conditions previously reported (Chenault et al. 2008). Reaction components: 10 µl (2.5 ng/µl) genomic DNA, 2 µl 10× PCR Buffer, 2 µl 25 mM $MgCl_2$, 1 µl each 10 µM Primers, 2 µl 2 mM dNTP mix, 0.5 µl Hot Start Taq Polymerase (5 U/µl), 1.5 µl H_2O. PCR products were visualized by electrophoresis in a 3.5% Metaphor agarose-TAE (Cambrex) gel at 130 V for 3 h and subsequent ethidium bromide staining. Bands were identified using Quantity One software (Biorad). Each banding pattern was verified by repeating reactions in triplicate. Resulting bands were scored as previously reported (Chenault et al. 2008). Genotypes possessing only the 145 bp band were given a score of *L*. When genotypes possessed both bands, those with a predominant 145 bp band were scored as *B* and those with a predominant 100 bp band were scored *b*. Finally, those genotypes carrying only the 100 bp band were given an *S* rating. Virginia-type accessions were analyzed as all others, but marker data obtained is for observation only and can not be used to predict *S. minor* resistance or susceptibility (Chenault et al. 2008).

Statistical analysis

For statistical analysis (Table 3), banding patterns were given numerical value: $L = 1$, $S = 2$, $B = 3$. The simple effects of marker given market type and the simple effects of market type given marker were evaluated with a SLICE option in an LSMEANS statement, and if a simple effect was significant at the 0.05 level, pair-wise comparisons of the levels of the factor in question were conducted with a DIFF option and adjusted using Tukey's procedure. Means and standard errors for the combinations of the factors are presented and letters used to represent the observed significant differences.

Results and discussion

Of the 112 accessions in the US peanut mini-core, a total of 96 were available and viable. Successful amplification was achieved for all available accessions using the primer pair specific for the molecular marker associated with Sclerotinia blight resistance in peanut. Of the four banding patterns possible upon amplification (*L*, *B*, *b*, and *S*), all but three accessions produced either the *L* or the *S* pattern. Three accessions produced pattern *B* (both bands present, larger band more intense). No accession produced

Table 1 List of available accessions of the US peanut mini-core collection included in this study and the results of molecular marker analysis

Core collection #	PI #	Country of origin	Market type	Marker score	Core collection #	PI #	Country of origin	Market type	Marker score
8	295730	India	Virginia	S	446	270905	Zambia	Mixed	L
12	493329	Argentina	Valencia	L	458	268996	Zambia	Runner	S
16	493356	Argentina	Virginia	L	468	270998	Zambia	Mixed	L
33	493547	Argentina	Valencia	L	477	268806	Zambia	Spanish	L
38	493581	Argentina	Valencia	L	481	268755	Zambia	Runner	L
41	493631	Argentina	Valencia	L	485	270786	Zambia	Mixed	L
47	493693	Argentina	Virginia	L	488	356004	Argentina	Mixed	L
50	493717	Argentina	Valencia	L	506	259658	Cuba	Runner	S
68	493880	Argentina	Valencia	L	508	259617	Cuba	Mixed	B
82	494034	Argentina	Spanish	L	516	288146	India	Virginia	L
87	475863	Argentina	Valencia	L	526	288210	India	Runner	L
97	497395	Bolivia	Virginia	L	529	319768	Israel	Virginia	L
112	497517	Brazil	Valencia	L	534	296550	Israel	Runner	S
115	496401	Burkina Faso	Virginia	L	535	296558	Israel	Runner	S
119	496448	Burkina Faso	Virginia	S	540	295250	Israel	Virginia	S
125	504614	Argentina	Mixed	S	541	295309	Israel	Mixed	S
132	497639	Ecuador	Valencia	B	542	370331	Israel	Virginia	L
149	502040	Peru	Spanish	L	546	259836	Malawi	Spanish	L
155	502111	Bolivia	Valencia	L	548	325943	Venezuela	Valencia	L
157	502120	Peru	Virginia	L	552	338338	Venezuela	Valencia	L
166	494795	Zambia	Runner	L	553	157542	China	Runner	S
187	331314	Argentina	Mixed	S	559	158854	China	Valencia	S
189	339960	Argentina	Valencia	L	579	271019	Zambia	Mixed	L
202	331297	Argentina	Mixed	S	580	268586	Zambia	Valencia	L
208	274193	Bolivia	Virginia	L	583	356008	Argentina	Valencia	L
221	290560	India	Spanish	L	588	403813	Argentina	Valencia	L
223	290620	Argentina	Virginia	L	610	475931	Bolivia	Virginia	L
227	290566	India	Runner	S	631	408743	Brazil	Mixed	S
230	290594	India	Runner	S	650	478819	India	Valencia	S
246	343398	Israel	Virginia	S	673	481795	Mozambique	Spanish	L
249	343384	Israel	Mixed	S	678	476636	Nigeria	Virginia	L
266	200441	Japan	Spanish	L	683	476596	Nigeria	Runner	S
270	196635	Madagascar	Runner	S	698	372305	Nigeria	Virginia	S
277	259851	Malawi	Virginia	S	703	476432	Mozambique	Mixed	L
287	355271	Mexico	Runner	S	725	240560	South Africa	Runner	L
294	372271	Nigeria	Virginia	S	728	292950	South Africa	Mixed	S
296	399581	Nigeria	Virginia	S	731	162857	Sudan	Virginia	S
310	337406	Paraguay	Runner	L	740	407667	Thailand	Spanish	L
334	159786	Senegal	Virginia	S	747	478850	Zambia	Valencia	B
338	268696	South Africa	Spanish	L	755	482189	Zimbabwe	Spanish	L
342	298854	South Africa	Runner	S	760	471952	Zimbabwe	Spanish	L
367	268868	Sudan	Virginia	S	763	442678	Zimbabwe	Virginia	S
381	313129	Taiwan	Mixed	S	775	482120	Zimbabwe	Spanish	L

Table 1 continued

Core collection #	PI #	Country of origin	Market type	Marker score	Core collection #	PI #	Country of origin	Market type	Marker score
384	155107	Uruguay	Valencia	L	781	471954	Zimbabwe	Valencia	L
388	162655	Uruguay	Spanish	L	787	429420	Zimbabwe	Valencia	L
406	152146	Uruguay	Spanish	L	798	461434	Nigeria	Runner	L
408	262038	Brazil	Valencia	L	802	196622	Cote D'Ivoire	Virginia	S
433	270907	Zambia	Mixed	L	812	323268	Pakistan	Virginia	S

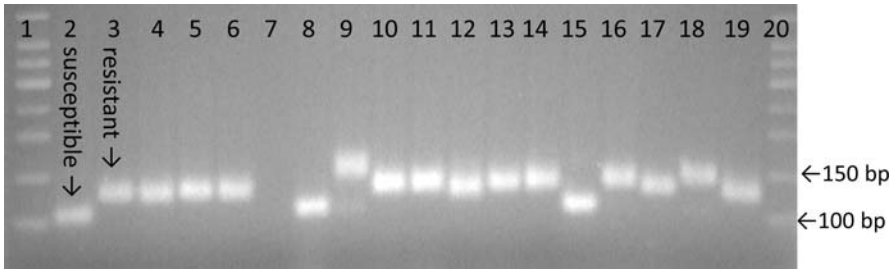

Fig. 1 Genotypic data typical of that collected for all available US peanut mini-core collection accessions. Lanes 1 & 20 = 50 bp ladder, 2 = Okrun, 3 = ARSOK-R1 (TX994313), 4 = PI 471952, 5 = PI 471954, 6 = PI 476432, 7 = PI 476636, 8 = PI 478819, 9 = PI 478850, 10 = PI 481795, 11 = PI 482120, 12 = PI 482189, 13 = PI 494795, 14 = 496401, 15 = PI 496448, 16 = PI 502040, 17 = PI 502111, 18 = PI 502120, 19 = PI 504614

pattern *b*. Data typical of such amplifications is shown in Fig. 1 and the genotyping result (marker score) for all accessions is listed in Table 1. Mini-core accessions have been well characterized phenotypically and the resulting descriptor data can be obtained from the Agricultural Research Service (ARS) Germplasm Resource Information Network (GRIN) database (http://www.ars-grin.gov), but market-type information for all accessions (Table 1) was either obtained from Kottapalli et al. (2007) or from C. Holbrook (personal communication). Eighteen (18) percent of the analyzed accessions were characterized as runner, 23% were Valencia, 27% were Virginia, 15% were Spanish, and 16% were described as a "mixed" or "intermediate" in market-type. A breakdown of marker score by market type is listed in Table 2.

Within the accessions evaluated, there are a surprisingly large number of accessions containing the large marker band associated with Sclerotinia blight resistance (approximately 60%), with the marker being especially predominant within the Spanish and Valencia accessions. In fact, 100% of all Spanish accessions tested produced the L banding pattern, supporting speculation that *Sclerotinia minor*

Table 2 Breakdown of marker analysis results by market type

Market type	Marker score			
	L	*B*	*b*	*S*
Runner	6	0	0	11
Spanish	14	0	0	0
Valencia	19	2	0	2
Virginia	12	0	0	14
Mixed	7	1	0	8
Total	58	3	0	35

resistance may have originally come from an introduction of a Spanish market-type peanut. The L banding pattern consistent with resistance is less dominant in the runner type accessions (35%) and more evenly distributed among the Virginia (46%) and mixed or intermediate (44%) market-types. No predictions are possible regarding the banding pattern produced by the Virginia market type accessions since the marker was previously shown not be significantly related to the resistance phenotype for Virginia cultivars (Chenault et al. 2008). As previously reported by Chenault et al. (2008), when

present, the marker consistent with a susceptible host response was fairly consistent in size at about 100 bp. The larger marker band which has been shown to be consistent with resistance varied in size with the most common being approximately 145 bp. Although all data is not shown, several accessions did produce single bands that were in the range of 165–175 bp, due to a longer repeat sequence in the amplified region (sequence data not shown), consistent with previously reported sequence data of cloned SSR marker fragments (Chenault et al. 2008; Barkley et al. 2007).

Upon statistical analysis, no significant correlation was seen between marker score and country of origin. There was, however, a significant relationship between market type and marker score (Table 3). The marker consistent with resistance (banding pattern $L = 1$) was more significantly related to Spanish and Valencia market type accessions than to the runner and mixed types. Again, although included in the analysis, no conclusions can be drawn from marker data obtained for Virginia introductions since there is no correlation between marker score and resistance level among Virginia market types.

Two mini-core collections of peanut germplasm have been created, one for the International Crops Research Institute for the Semi-Arid Tropics or ICRISAT (Upadhyaya et al. 2002) and the other for the US (Holbrook and Dong 2005) and both have been phenotypically characterized using a set of standard descriptors. Some limited characterization of the collections has been done using molecular tools. Kottapalli et al. (2007) used microsatellite (SSR) markers to examine 72 accessions of the US mini-core collection and were able to group the accessions by subspecies and market class using molecular data. The genetic diversity of cultivated and wild species in the US mini-core was also assessed by Barkley et al. (2007) using M13-tailed SSR markers, confirming much of the taxonomic classification of the collection. Chu et al. (2007a, b) reported on the US mini-core's frequency of a loss-of-function mutation in oleoyl-PC desaturase (*ah-FAD2A*), the mutation which results in a dysfunctional desaturase and subsequent high-oleic acid content of peanut seed. Finally, Kang et al. (2007) used sodium dodecyl sulfate-polyacrylamide gel electrophoresis to describe the distribution of three peanut allergens among members of the US mini-core collection. Although the mini-core has been examined phenotypically for resistance to diseases such as leaf spot (Holbrook and Anderson 1995), no studies documenting the distribution of Sclerotinia blight resistance have been reported for the collection.

Previously, the marker used in this study was shown to be significantly associated with resistance to Sclerotinia blight in peanut cultivars and PIs that had been thoroughly evaluated in field trials (Chenault et al. 2008). In this study, 39 accessions spanning runner, Valencia and Spanish market-types were identified as potential new sources of Sclerotinia blight resistance. Since no reports of field evaluation of the mini-core collection for Sclerotinia blight resistance or susceptibility are available to aid in further validation of this marker as a selection tool for breeding programs, field trials are currently underway toward that end. However, the results obtained in this study have identified PIs worthy of such evaluation in the field for *S. minor* resistance and also identified those PIs with low probability of being a source of resistance, reducing the amount of field work required to test the mini-core collection by 34% for the runner, Valencia and Spanish market type accessions.

Table 3 Correlation between market type and marker score using Tukey's adjustment

Market type	Mean	Standard error
Spanish	1.00a	0.00
Valencia	1.26ab	0.13
Runner	1.65b	0.12
Virginia	1.54b	0.10
Mixed	1.63b	0.15

Numbers followed by the same letters are not significantly different

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